

AD _____

Award Number: W81XWH-04-1-0643

TITLE: Cellular Plasticity of Epithelial Cells-Cause of Metastasis

PRINCIPAL INVESTIGATOR: Saraswati Sukumar, Ph.D.

CONTRACTING ORGANIZATION: John Hopkins University School of Medicine
Baltimore, MD 21205

REPORT DATE: September 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 01-09-2005	2. REPORT TYPE Final	3. DATES COVERED (From - To) 6 Aug 2004 – 5 Aug 2005		
4. TITLE AND SUBTITLE Cellular Plasticity of Epithelial Cells-Cause of Metastasis		5a. CONTRACT NUMBER		
		5b. GRANT NUMBER W81XWH-04-1-0643		
		5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Saraswati Sukumar, Ph.D. E-mail: sukumsa@jhmi.edu		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) John Hopkins University School of Medicine Baltimore, MD 21205		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT Metastasis is a major factor contributing to casualties in cancer patients. In this award, we sought to address critical questions as to how the rare metastatic epithelial cells can find and contact the much rarer angiogenic endothelial cells, or how a new angiogenic network is setup. We present a novel concept that cancer epithelial cells, possibly of stem cell origin, have inherent cellular plasticity and can differentiate into endothelial cells and form microvessels that serve as a conduit for entry of epithelial cells. To test this hypothesis we marked metastatic breast cancer cells with GFP and looked for expression of endothelial markers in the green epithelial cells.				
15. SUBJECT TERMS No subject terms provided.				
16. SECURITY CLASSIFICATION OF: a. REPORT U b. ABSTRACT U c. THIS PAGE U		17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 6	19a. NAME OF RESPONSIBLE PERSON USAMRMC
				19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusions.....	6

Concept award:

Principal Investigator: Saraswati Sukumar, PhD

Metastasis is a major factor contributing to casualties in cancer patients. However, critical questions as to how the rare metastatic epithelial cells can find and contact the much rarer angiogenic endothelial cells, or how a new angiogenic network is setup, are rarely addressed. We present a novel concept that cancer epithelial cells, possibly of stem cell origin, have inherent cellular plasticity and can differentiate into endothelial cells and form microvessels that serve as a conduit for entry of epithelial cells into the circulation.

To test this hypothesis we undertook the following steps.

Statement of Work:

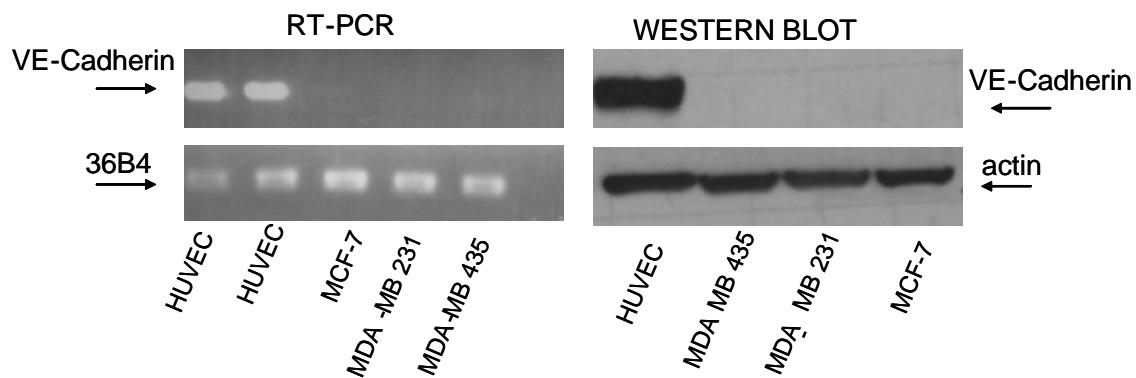
These experiments will address the question of epithelial cell plasticity and function in the breast cancer metastatic process.

Months 1-3:

Task 1: Breast cancer cell lines, MDAMB231 and MDAMB468 will be stably transfected with enhanced green fluorescence protein (GFP)-marker, selected using G418 and stable clones will be generated that have strong expression of EGFP.

To test this hypothesis, we first needed to label the epithelial cancer cells with GFP and inject the cells into mouse mammary glands. Thereby, we could track these cells and check whether these cells can express the endothelial specific markers *in vivo*. Thus, we established stable pool and single clones of GFP-labeled non-metastatic MCF-7 cells, metastatic MDA-MB 231 and MDA-MB 435 cells.

Since our goal is to demonstrate epithelial to endothelial transition *in vivo*, we first used RT-PCR and western blot to ensure that no endothelial specific marker, VE-Cadherin, was expressed in these cells. (see figure below)



Months 4-6

Task 2: A million tumor cells from a stable clone with strong EGFP expression of the two cell lines will be injected into groups of 6 SCID mice to grow as tumors in the fat pad. Two- three weeks later, when the tumors are about 10 mm in diameter, the tumors will be excised.

These cells were injected into nude mice both by intramammary gland injection and intracardiac injection. After 8 weeks, both *in situ* and metastatic tumors to lung and liver were recovered and fixed with formalin.

Months 6-8

Task 3: Immunostaining of the tumor fixed in formalin, or preserved as frozen tissues will be performed. Anti-GFP antibody, and endothelial specific antibodies (PH12, von Willebrand factor, VE-cadherin) will be used to study expression in blood vessels and tumor cells.

Tumors recovered from the mice have been paraffin embedded. Immunofluorescent staining on these samples will be used to check whether these green cancer epithelial cells will express VE-Cadherin. If so, we will also check whether VE-Cadherin expression will be different between non-metastatic and metastatic cells.

Months 5-8:

Task 4: The tumor pieces from Task 3 will be serially passaged three times as xenografts in the SCID mouse mammary fat pad. We will perform staining for markers of endothelium, to see if the tumor cell populations have an increasing proportion of cells expressing endothelial markers in later passages.

Tumor pieces have been transplanted for serial passage. Further passage tumors will be collected for examination of endothelial markers.

Months 8-12

Task 4: We will construct a vector with expression of Herpes thymidine kinase (HTK), a drug susceptibility gene, under the control of endothelial specific promoter, VE-cadherin. This construct will be transfected into MDAMB231 and stable clones will be derived by G418 selection.

Task 5: Cells will be grown as tumors in the mouse mammary fat pad (as in task 1), and treated with acyclovir or ganciclovir (2X a day for 5 days). Cells with endothelial transformation alone will die. If this epithelial to endothelial transformation is critical for metastasis, mice treated with drug will show significantly lower metastasis than the control.

Task 6: Evaluate results. Plan new detailed experiments if the results reflect the hypothesis.

Tasks 5 and 6 will be undertaken as soon as clones of MDAMB 231 cells are ready.

Key Research Accomplishments:

- 1: Established cell lines expressing GFP
- 2: Xenograft assays were done.
3. Passage of xenograft assays ongoing.

Reportable Outcomes

Lianfeng Han's presentation at Era of Hope conference of abstract entitled "Cellular Plasticity of

Epithelial Cells-Cause of Metastasis”.

Conclusions

Work is ongoing and conclusions will be drawn after data has been analyzed.

References

None

Appendices

None